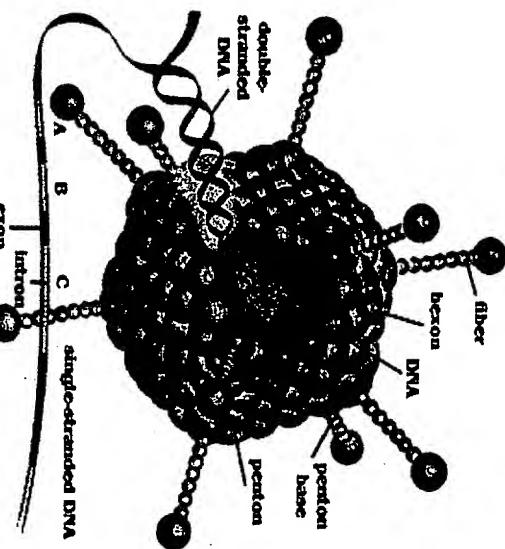


Crucell

Patent application 09/348,354  
“Chimeric adenoviruses”



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# Modified vector library

Adenovirus type 5

Transgenic

FIBER



Library of Fibers

PCR amplify



*Production on PER.C6*

Adenovirus type 5

Transgenic

FIBER

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# **Definition of "Tropism"**

**ent (US6,127,525):**

**Specificity or natural affinity for certain tissues  
organism".**

- # Definition is limited to binding of virus to cell type due to compatibility of virus and cellular receptor.

**cell.**

**The sum of biological processes that determine whether  
virus can transfer a gene in vivo to a particular cell,  
organ or tissue.**

- # Anatomical barriers
- # Viral lysis by serum components (non-antibody related)
- # Neutralizing antibodies
- # Receptor-virus compatibility
- # Vector stability

# R. Anatomical barriers

For instance vessel wall barrier the virus is  
unable to reach the target of interest.

Due to the size of Adenovirus, penetration in tumor  
tissue is severely limited.

Expression profile of Ad5 receptor does not correlate  
with observed Ad5 infection patterns in rodents

(Fechner et al Gene Ther. 1999 Sep;6(9):1520-35)

# viral lysis by serum

monkey (pre-treatment)

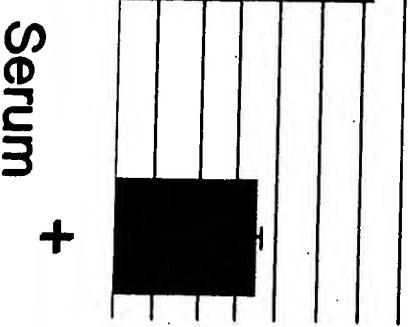
A549

Luciferase act.

1000000  
100000  
10000  
1000  
100  
10

1

- Serum +



1/4 diluted serum, dose: 500 vp/ cell, A549 cells

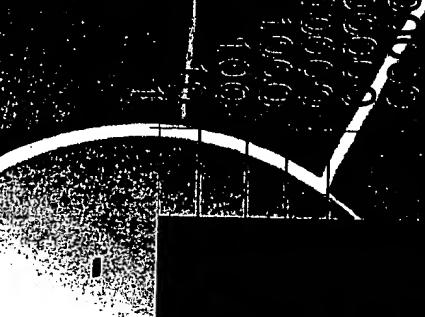
Serum effect is independent of antibodies

Luciferase activity

100000  
10000  
1000  
100  
10

1

- Serum +



Cynomolgus

Rhesus

• Crucell

# Patent (6,127,525): Concerning fiber swap and *In vivo* escape of Nab

Column 3 (columns 25)

These results confirm that switching the fiber from that of adenoviral serotype 5 vector to that of an adenoviral serotype 7 subgroup B vector by itself is sufficient to allow the vector to escape neutralising antibodies generated against an adenoviral vector comprising Ad5 fiber.....”

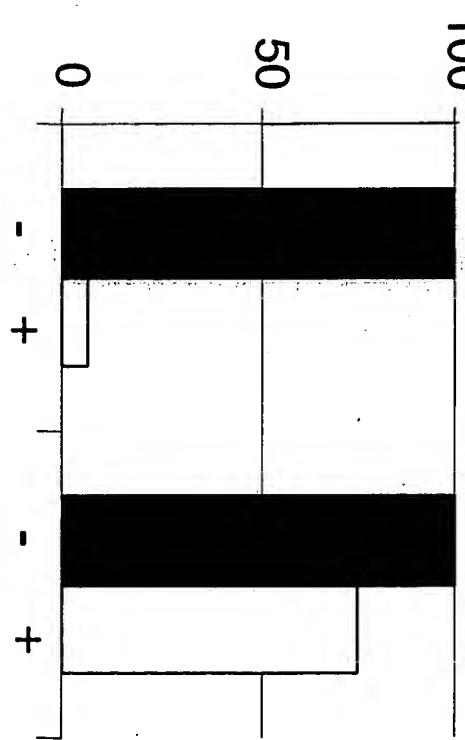
# Neutralizing antibodies

C3H mice

Not significant

p=0.008

Not significant

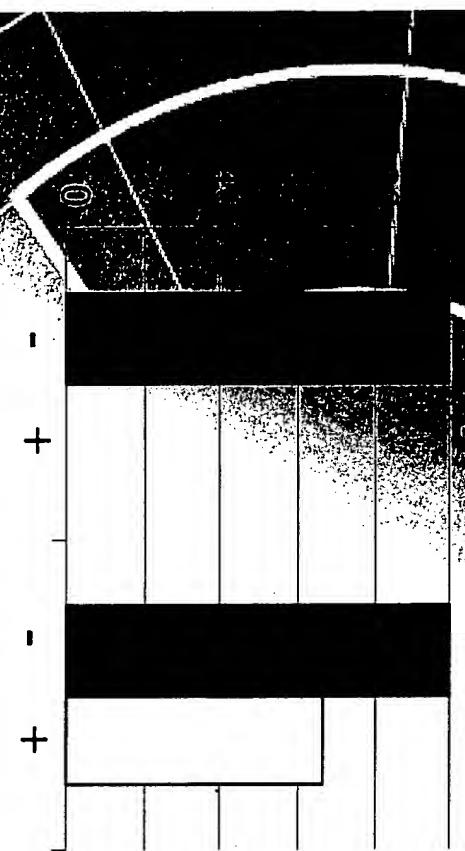


C3H mice

Not significant

p=0.008

Not significant

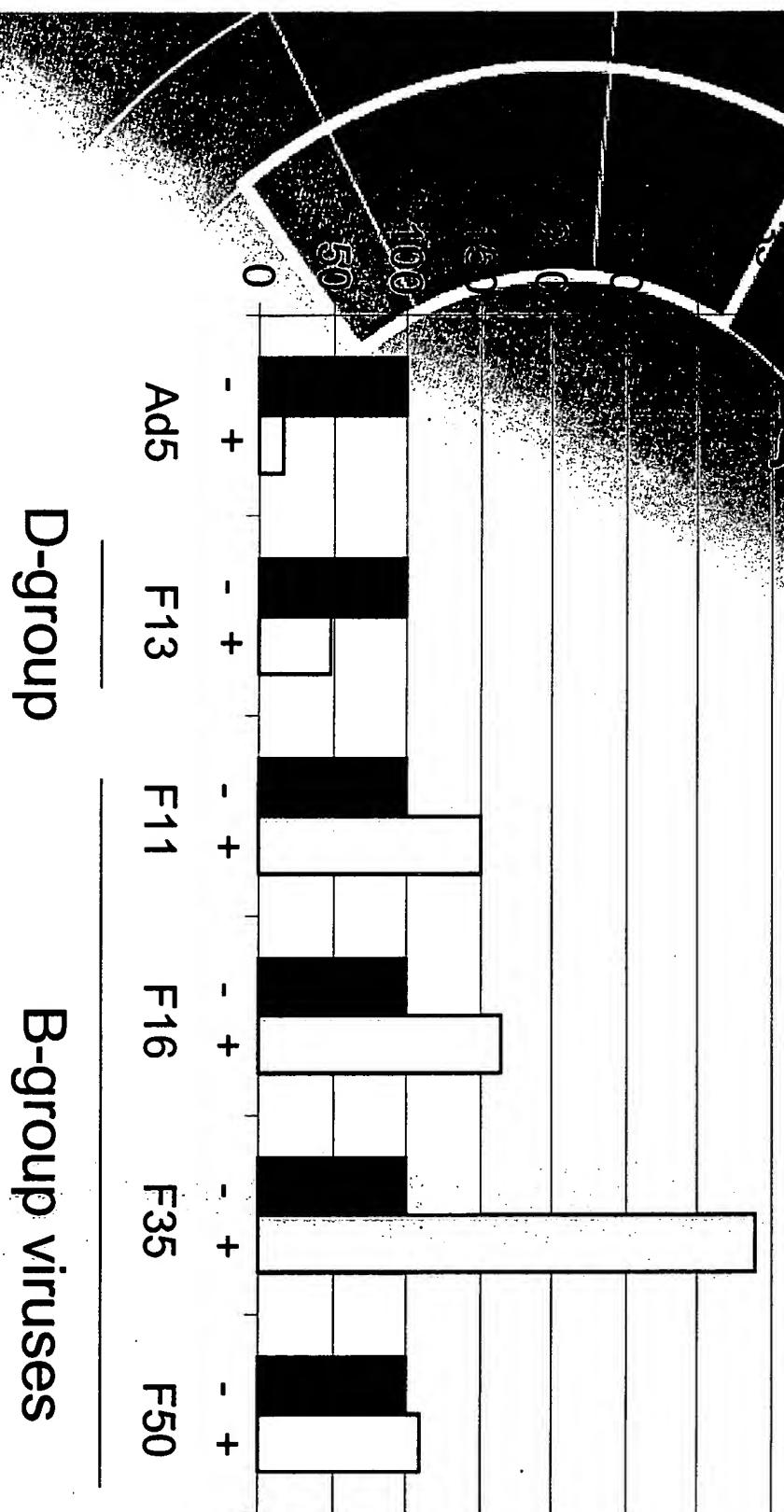


P values determined via "Mann-Whitney U" test

• Crucell

# Neutralizing antibodies

C57/ Bl6 mice



P values determined via "Mann Whitney U test"

• Crucell

# Changes in structural design of fiber-chimeric vector

• **Blattner**

~ Deletion of Ad5 fiber and insertion of complete Ad7 fiber.

• **Staib, J. Virol Vol 70, p2120:**

“...we have shown that amino acid homology between the tail regions of Ad5 and Ad7 is sufficient to allow functional replacement of the Ad5 fiber with Ad7 fiber....”

• **Crucell:**

~ Retained Fiber tail of Ad5 to ensure proper interaction with Ad5 penton-base  
(i.e. homology between Ad7 and Ad5 in fiber tail region is 57% on a.a. level)

Substantial difference in vector stability expected

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